# OSMOTIC ADJUSTMENT IN *PINUS TAEDA* FAMILIES IN RESPONSE TO WATER STRESS

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*Abstract*--Osmotic adjustment is often critical for seedling survival under drought stress. This study examined: (1) the potential of cyclic soil water deficits to induce osmotic adjustment in *Pinus taeda* L. seedlings from two half-sib families, the one from a xeric provenance, the other a mesic, and ($^2$) the influence of these water deficits on cell wall elasticity and symplasmic volume. Seedlings were grown for five months under low stress conditions in an environmental chamber. Water deficits were induced by withholding soil water. Predawn xlyem water potentials did not exceed -0.6 MPa in the daily watered control treatment while in the stress treatment, seedlings were watered to saturation only when predawn xylem potentials reached -1.5 MPa (once/12-13 days). After four stress cycles, psychrometric analysis indicated a significant osmotic adjustment of -0.4$^2$ MPa relative to the controls. There was no significant difference in adjustment between families. Pressure-volume curve analysis confirmed significant adjustment and indicated that cell wall elasticity and symplasmic volume declined significantly due to stress. Results suggest osmotic adjustment is a common mechanism of drought resistance in *Pinus taeda*.

*Additional keywords*: loblolly pine, cell wall elasticity, pressure-volume, symplasmic volume

## INTRODUCTION

The maintenance of cell tugor as soil moisture and/or plant water potential (T) declines is critical for normal cell function and plant survival. Stomatal closure, photosynthesis, cell expansive growth and cell division are highly sensitive to turgor potential ($gy_p$) ( Turner and Jones 1980, Bradford and Hsiao 1982). A number of physiological mechanisms interact to maintain W. Among them osmotic adjustment, because of its fundamental position as a water potential component, has received the most attention (e.g. Hinckley et al. 1980, Meinzer et al. 1986, Sobrado 1986, Joly and Zaerr 1987). Osmotic adjustment is a decrease in the symplastic osmotic potential (T.) (Parker and Pallardy 1985); it results from an increase in solute concentration within the symplasm. The adjustment can be caused by an *active* net accumulation of solutes in the symplasm or alternatively, a *passive* increase in solute concentration due to tissue dehydration or change in partitioning of water between the symplasm and apoplasm (Joly and Zaerr 1987). A decline in TW caused by the first mechanism is true adjustment (Hinckley et al. 1980).

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While osmotic adjustment is a normal process in many woody plants (Bowman and Roberts 1985, Meinzer et al. 1986, Sobrado 1986) it has attracted recent attention because of the need to increase early drought resistance of outplanted tree seedlings. If seedling osmotic adjustment can be enhanced by sublethal preconditioning with drought stress then survival may be increased (Parker and Pallardy 1985, Seiler and Johnson 1985, Joly and Zaerr 1987).

*Pinus taeda* is the most important commercial pine species in Texas and the southeastern United States. In Texas and the Western Gulf drought stress is the single greatest cause of seedling mortality (Karr et al. 1984). It has been demonstrated that in *Pinus taeda*, preconditioning drought stress can induce at least apparent osmotic adjustment (Hennessey and Dougherty 1984, Seiler and Johnson 1985). Yet, preliminary work has indicated that rapidly induced severe drought stress may result in minimal osmotic adjustment (Wilson 1985) and there may be major differences between families of *Pinus taeda* in ability to osmotically adjust (Holmes 1986).

The objective of this study was to evaluate the potential for inducing osmotic adjustment by cyclical slow development of high sublethal water deficits and to examine the ability of contrasting *Pinus taeda* families, to osmotically adjust.

## METHODS

### Plant materials and growth conditions

Seedlings were grown from seed collected in seed orchards from two half-sib families. The one family (GR1-8), provided by the Texas Forest Service, originated from the western edge of the species natural range and is known for superior early survival and growth on xeric sites. The other (8-76), provided by Weyerhaeuser Co. originated from the North Carolina Coastal Plain and is known for its superior early growth on mesic sites.

Plants were grown in one liter - Rootainers ; (Spencer-Lemaire Industries, Ltd.) filled with fritted-clay. This media was selected because it retains large amounts of water relatively uniformly absorbed over the -0.1 to -1.5 MPa range of soil water potentials and has excellent aeration characteristics (van Bavel et al. 1978). Seedlings were watered daily. For five days of each week they received 85 ml of a fertilizer solution developed for pine seedlings (Miller 1982). Then, for the remaining two days all containers were watered to saturation with distilled water to limit salt build-up.

Seedlings were grown in a controlled environment chamber during the entire experiment. Temperature and relative humidity were maintained at 25 ± 1 °C and 87 ± 5%, respectively. Day length was 16 hours with 640 uE $m^{-2}$ $sec^{-2}$ of photosynthetically active radiation at plant height. Seedlings were rotated within the chamber to assure uniform growth environment.

### Drought Stress Treatment and Measurement Methods

After five months seedlings were randomly assigned to control and drought stress treatments. All fertilization was stopped. The control treatment received 90 ml of distilled water daily. In the stress treatment water was withheld until predawn water potentials reached = -1.5 MPa. Then all seedlings were watered to saturation for two days. Predawn fascicle water potentials were determined daily for all treatment - seed source combinations using the pressure chamber technique (Ritchie and Hinckley 1975) with the aid of a dissecting microscope (40x) to detect pressure balance points. After each drought stress cycle, seedlings from each treatment seed source combination were randomly selected for analysis of osmotic adjustments (Seiler and Johnson 1985). In the first two cycles five seedlings per family-treatment combination were analyzed. This number was increased to 10 in later cycles.

The root systems of selected seedlings were immersed in distilled water and seedling shoots were covered with plastic bags. Contact between the foliage and bag was minimized. Seedlings were then allowed to rehydrate at 3 ± .5°C for 36 to 48 hours. After rehydration the water potentials (T) of two fascicles per seedling were determined by use of previously described pressure chamber methodology. Simultaneously, two mature needles were taken from each seedling for measurement of osmotic potential. Needles were frozen in liquid nitrogen and osmotic potential was determined psychrometrically (Wescor, Inc., models HR33T, C-52, Logan UT) using the expressed sap method of Turner et al. (1978). Measurements were not corrected for non-symplasmic water. Turgor potentials were calculated as: $T_p$ – T - Tpi.

After apparent osmotic adjustment had been detected by the above technique seedlings were subjected to an additonal treatment cycle. Then bulk shoot water relations were characterized from pressure-volume curves (Tyree and Hammel 1972) for shoots hydrated as previously described. Osmotic potentials at full saturation (',$_o$) and the turgor loss point (T,$_p$), symplastic water fraction (SWF) and relative water content at the turgor loss point (RWC $_p$) were estimated using methods described by Cheung et al. (1975). Maximum bulk modulus of elasticity $_{(Emax)}$ was derived following the method of Bowman and Roberts (1985). Ten seedlings per family-treatment combination were analyzed.

## RESULTS

In the stress treatment predawn xylem water potentials were higher (Figure 1) in the GR 1-8 family and declined at a slower rate than in the 8-76 family. This difference was primarily due to the smaller size and thus smaller transpiring surface of the GR1-8 family (Table 1). There was no significant difference between families in mid-day stomatal resistance (unpublished data), which might also have been a factor. As shown in Table 1 the 8-76 family showed a more rapid growth rate before treatment and, even under stress, nearly doubled in shoot mass during the stress treatment period. The GR1-8 family exhibited a consistently more conservative growth rate.

After four drought stress cycles significant differences in water potential components were observed between families and treatments (Table 2). Rehydration of seedlings prior to measurement increased xylem water potentials, but stressed seedlings still had significantly, slightly lower (-0.10 MPa) 1Y values (Table 2). In both families W,. values were also significantly influenced by the cyclic moisture stress treatment, averaging a near identical -0.43 MPa lower in the rehydrated stressed seedlings. Because of the decreased $T_{pi}$ values in the seedlings preconditioned by cyclic stress, ^$Y_p$ values were significantly higher than in the control treatments; the two families again averaged a near identical, significant increase of 0.34 MPa due to treatment.

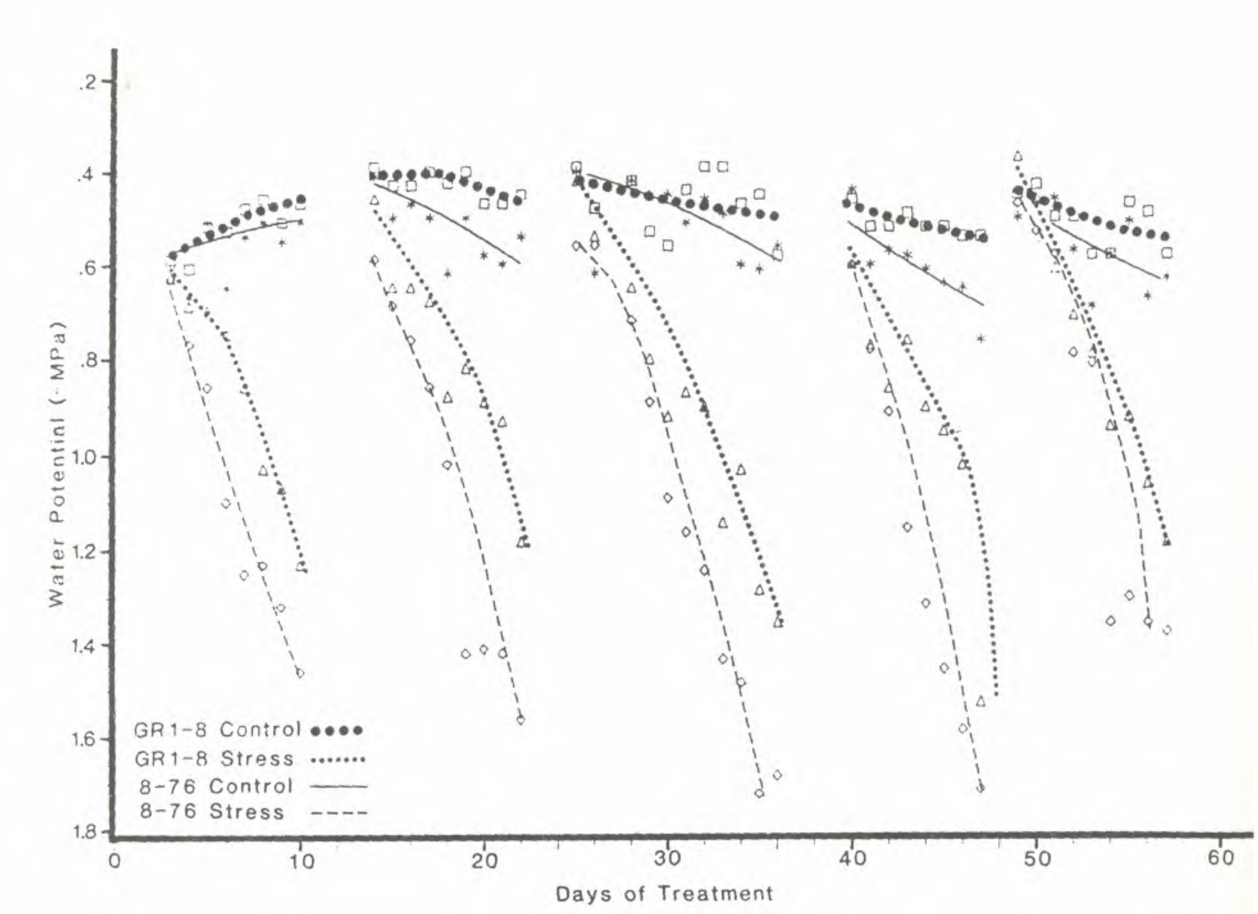
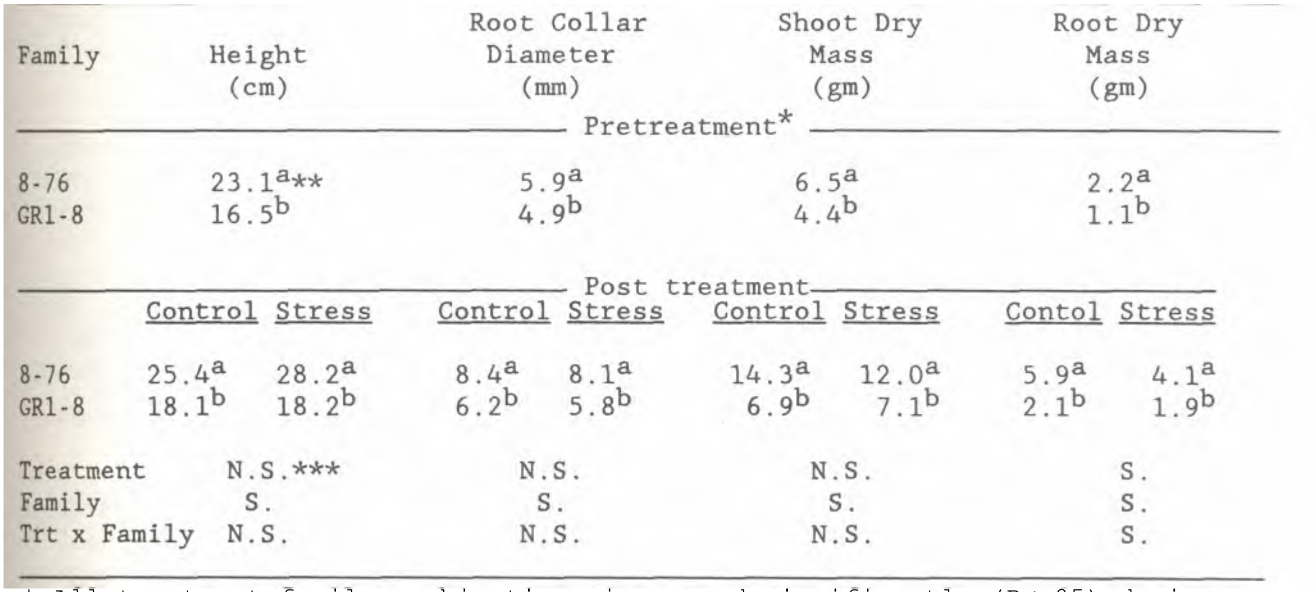


Figure 1. Predawn needle water potentials of loblolly pine seedlings from two families (GR1-8, 8-76) under control (daily watered) and stress (water withheld) treatments. All treatments watered to saturation when stress treatment reached approximately = -1.5 MPa.

All T, Tpi, and Tpi values for family 8-76 differed significantly from their treatment counterparts in GR1-8 (Table 2). Differences between families in T while statistically significant, were minimal, averaging -0.09 MPa lower in the 8-76 family. Osmotic and turgor potentials differed by an average of -0.21 and 0.12 MPa between families with the 8-76 having the lower and higher value, respectively. It should be noted that pattern of difference in $*_p$ relative to T and Tpi was predictable because of the way $T_p$ was estimated.

To provide confirmation and to elucidate the above trends, pressure-volume curve analyses were performed. In both families, osmotic potentials at full saturation were significantly lower in the seedlings preconditioned by cyclic stress (Table 3). However, Tpi*o values for control and stressed seedlings from the 8-76 family were significantly lower than those of counterpart seedlings in the GR1-8 family by -0.1 and -0.24 MPa, respectively. Comparison of **T**,$_{pi*o}$ values (Table 3) with W,, values near saturation (Table 2) indicates that psychrometrically determined values, are as expected lower and differences between treatments more pronounced in the latter measurements. These differences reflect differences in degree of rehydration between treatments and families and analytical methods. The latter is supported by practical and theoretical considerations (Ritchie and Dunham 1979) and the former by observed variance between treatments and families in Tpi*o (Table 3). However, the concurrence of pattern in significant differences between families and treatments supports the validity of osmotic adjustment estimated psychrometrically (Table 2).

Table 1. Comparison of Seedling growth characteristics of two Pinus taeda families under drought stress treatments.



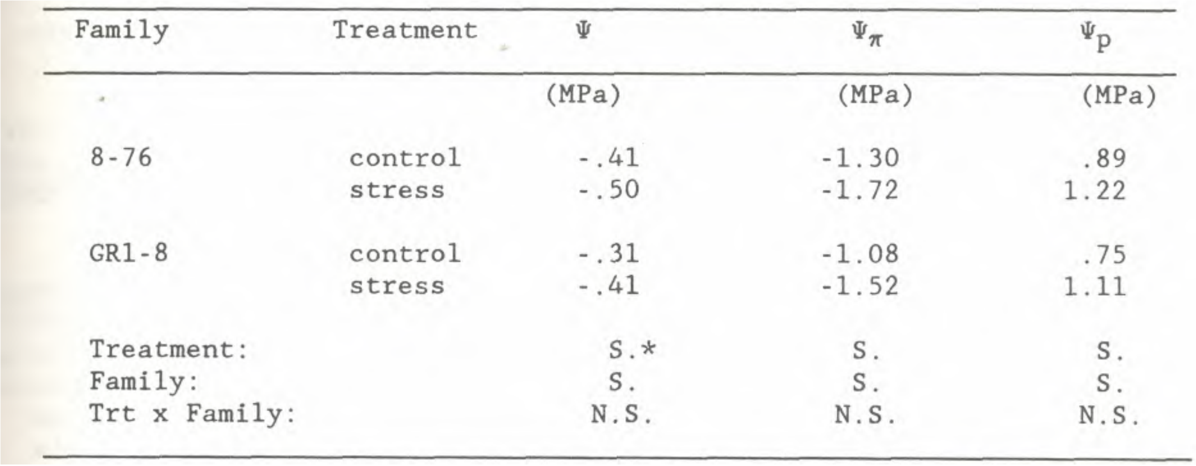
| Family | Height (cm) | | Root Collar Diameter (mm) | | Shoot Dry Mass (gm) | | Root Dry Mass (gm) | |
|---|---|---|---|---|---|---|---|---|
| Pretreatment* | | | | | | | | |
| 8-76 | 23.1$^{a}$** | | 5.9$^{a}$ | | 6.5$^{a}$ | | 2.2$^{a}$ | |
| GR1-8 | 16.5$^{b}$ | | 4.9$^{b}$ | | 4.4$^{b}$ | | 1.1$^{b}$ | |
| Post treatment | | | | | | | | |
| | Control | Stress | Control | Stress | Control | Stress | Contol | Stress |
| 8-76 | 25.4$^{a}$ | 28.2$^{a}$ | 8.4$^{a}$ | 8.1$^{a}$ | 14.3$^{a}$ | 12.0$^{a}$ | 5.9$^{a}$ | 4.1$^{a}$ |
| GR1-8 | 18.1$^{b}$ | 18.2$^{b}$ | 6.2$^{b}$ | 5.8$^{b}$ | 6.9$^{b}$ | 7.1$^{b}$ | 2.1$^{b}$ | 1.9$^{b}$ |
| Treatment | N.S.*** | | N.S. | | N.S. | | S. | |
| Family | S. | | S. | | S. | | S. | |
| Trt x Family | N.S. | | N.S. | | N.S. | | S. | |

* All treatment-family combinations increased significantly (P<.05) during treatment.

** Statistically significant differences (P<.05) between families within measurement periods are noted by different superscript letters.

***S. and N.S. denotes significance and nonsignificance, respectively at the 0.05 level.

Table 2. Needle water (41), osmotic (Tpi ), and turgor (T $_{pi}$) potentials in Pinus taeda families as influenced by cyclic drought stress.



| Family | Treatment | $\Psi$ | $\Psi_{\pi}$ | $\Psi_{p}$ |
|---|---|---|---|---|
| | | (MPa) | (MPa) | (MPa) |
| 8-76 | control | -.41 | -1.30 | .89 |
| | stress | -.50 | -1.72 | 1.22 |
| GR1-8 | control | -.31 | -1.08 | .75 |
| | stress | -.41 | -1.52 | 1.11 |
| Treatment: | | S.* | S. | S. |
| Family: | | S. | S. | S. |
| Trt x Family: | | N.S. | N.S. | N.S. |

*S. and N.S. denote significance and non-significance, respectively, at the 0.05 level.

Table 3. Comparison of osmotic potential at full saturation (f $_{ro}$), osmotic potential at turgor loss point (Tpi*p), bulk molulus of elasticity ($_{E}$max)' relative water content at turgor loss point (RWC$_p$) and symplasmic water fraction (SWF) between drought stress levels and two families of *Pinus taeda*.

| Family | Treatment | $\Psi_{\pi o}$ | $\Psi_{\pi p}$ | $\xi_{max}$ | RWC$_p$ | SWF |
|---|---|---|---|---|---|---|
| | | (MPa) | (MPa) | (MPa) | | |
| 8-76 | control | -1.38 | -1.95 | 10.6 | .90 | .36 |
| | stress | -1.60 | -2.18 | 17.3 | .92 | .32 |
| GR1-8 | control | -1.28 | -1.89 | 12.0 | .89 | .36 |
| | stress | -1.40 | -2.09 | 21.2 | .91 | .27 |
| Treatment: | | S.* | S. | S. | S. | S. |
| Family: | | S. | N.S. | N.S. | N.S. | N.S. |
| Trt x Family: | | N.S. | N.S. | N.S. | N.S. | N.S. |

*S. and N.S. denote significance and non-significance, respectively, at the 0.05 level except for SWF which is at the 0.08 level.

In the other water relations parameters measured (Table 3) there was not a significant difference between families. However, within families cell wall elasticity decreased (higher $_{Amax)}$ significantly due to the stress treatment; RWCp showed a slight but significant increase in the seedlings subjected to cyclic stress; Tpi*p declined in seedlings under stress; and the decline in SWF under stress was significant at the 0.08 level.

## DISCUSSION

Evaluation of tissue water relations by both psychrometric and pressure-volume methods have indicated significant osmotic adjustment, due to water stress. The appoximately 0.4 MPa decrease in It. of stressed seedlings, (Table 2), would allow for maintenance of turgor to needle water potentials approximately 0.4 MPa lower then controls. Stressed seedlings had 0.10 MPa lower T values with T$_p$ values approximately 0.34 MPa higher than control seedlings. These magnitudes are similar to those reported by Seiler and Johnson (1985) for a single *Pinus taeda* source. The T$_{wo}$ values (Table 3) are similar in magnitude to those reported by Hennessey and Dougherty (1984), and magnitudes of adjustment, especially of the 8-76 family, agree well with their results.

In comparing levels of stress, it should be noted that the cyclic stress which Seiler and Johnson (1985) imposed approximated the level in this study; however, the cyclic stress which Hennessey and Dougherty (1984) imposed had a minimum value of -7.5 MPa. Thus, their stress treatment was roughly equivalent to our control treatment in minimum xylem water potential. For this reason, magnitudes of osmotic adjustment reported here may be underestimates of the total osmotic adjustments which stressed seedlings undergo relative to minimally stressed individuals.

Osmotic adjustment in the stressed seedlings appears due to both active and passive process. Declines in T$_{iro}$ of stressed seedlings can be partially explained by decreases in SWF, a passive adjustment process; however, the magnitudes of osmotic adjustment (Table 2, 3) strongly suggest an active process is also present.

Decreases in SWF are often associated with production of cells of decreasing size and increased cell wall thickness by plants under drought stress. This explanation is in agreement with the significant growth which occurred during treatment and supported by the higher $_{\text{£max}}$ and RWC in stressed seedlings, both of which are indicative of decreased cell wall elasticity (Parker and Pallardy 1985).

In summary there is a significant decrease in Tpi*p Tp$_{i*p}$ for the preconditioned seedlings. Thus, osmotic adjustment has occurred. While it appears that both active and passive processes are interacting in the development of the observed osmotic adjustment the presence of true osmotic asjustment appears evident. This combination of factors, a high $_{\text{Amax}}$ and a low Tpi (large osmotic adjustment) as pointed out by Bowman and Roberts (1985) is not in conflict. Together they can create large relatively rapid decreases in symplasmic water potential which facilitates continued water uptake while maintaining positive turgor and cell expansive growth.

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